EARLY DEVELOPMENT OF GRAFFILLA GEMELLIPARA —A SUPPOSED CASE OF POLYEMBRYONY.[1]

J. THOMAS PATTERSON.

I. Introduction.

In the Brooks' Memorial Volume of the *Journal of Experimental Zoölogy*, Vol. 9, 1910, Professor Edwin Linton reports the discovery of a very interesting viviparous rhabdocœle commensal with the common ribbed mussel, *Modiolus plicatulus*, found along the Atlantic coast. Dr. Linton refers this worm to the genus *Graffilla*, and on account of its peculiar method of producing embryos in pairs, designates it by the name *Graffilla gemellipara*. So far as we know the only other statement in the literature that could be interpreted as referring to this interesting turbellarian is found in a short paper by Nicoll, '06, entitled "Notes on Trematode Parasites of the Cockle (*Cardium edule*) and Mussel (*Mytilus edulis*)."

Nicoll figures (in his Fig. 7) what he calls a trematode sporocyst from the liver of the cockle, but it is quite clear from Linton's work that he is in error in calling this specimen a sporocyst. What he in all probability had was a specimen of a species of turbellarian closely related to if not identical with *G. gemellipara*. This is evident from the fact that his figure shows the presence of paired embryos, as well as a pharynx, which alone would exclude the case from the category of sporocysts.

Linton's paper gives an account of the more general features of the worm, but leaves several important questions unanswered, among which may be mentioned the following: (1) How is the yolk deposited in the ova? (2) How do the sperms reach the "sperm-sac"? (3) Is the species protandrous? (4) Where are the eggs fertilized? (5) Finally, and most important of all, How do the two embryos in each capsule arise?

In regard to this last point, Linton suggests that we may have a

[1] From the Marine Biological Laboratory, and the Zoölogical Laboratory of the University of Texas. Contribution No. 109.

case of polyembryony. It was this suggestion that induced me to undertake a study of certain phases of the development of *Graffilla;* and this not only because of my interest in the general subject of polyembryony, but also for the reason that an opportunity seemed to be offered to work out the details of this peculiar phenomenon. Furthermore, if a true gemelliparous development really did exist in so simple a fashion in a relatively low organism like *Graffilla*, it might be possible to modify experimentally the process and thus to be able to get at some of the factors underlying it.

While the results obtained from these studies have proved disappointing, at least so far as the main object for which the investigation was undertaken, yet they are of a character such as to warrant record, especially as they answer satisfactorily some of the questions raised above. Furthermore, we have as yet only a very few papers dealing with the development of rhabdocœles, and consequently there is need of contributions along this line.

Methods.—Various methods for preserving the material have been used, but the most successful fixing fluid has been found to be Benda's modification of Flemming's strong solution. Specimens fixed for two hours in this fluid give beautiful results for cytological study, especially when followed by iron-hæmatoxylin stains. Bouin's fluid also gave good preparations, but is much less certain in its results. In making whole mounts the specimens are placed under slight pressure and killed over a gentle flame, and then fixed in a corrosive-sublimate solution. If followed by borax carmine such material gives very clear figures of many structures. However, I find the same "indefiniteness" about the reproductive organs as noted by Linton, especially in regard to the ducts, so that one can not rely upon mounts for one's interpretation of the conditions of these structures.

Notes on the Habits.—Linton states that *G. gemellipara* lives on the gills of *Modiolus*, but there is some evidence that they inhabit the kidney. This is brought out in the following experiment. Two dozen specimens of *Modiolus* from a lot yielding no *Graffillæ* from the gills were opened, care being taken not to injure any of the tissues, and thoroughly washed out in water. No parasite was found. The kidneys of these same individuals

were teased out and the specimens again washed in water, with the result that thirty-eight *Graffillæ* were secured. Undoubtedly many individual parasites escape from the kidney of the host and are later found in the mantle cavity and on the gills, and this would account for their discovery there by Linton. Furthermore the method ordinarily employed in opening the molluscs would necessarily result in injuring the kidney, and thus permit the escape of the parasite from that organ. The experiment mentioned above would seem to indicate clearly that *G. gemellipara* is a true endoparasite, but the experiment was performed at the close of the season and the opportunity was not offered to settle the question conclusively, as that could only be done by making careful dissections of the individual molluscs. We should expect to find this species of parasite in the kidney or liver of the host since all of the other species of the genus *Graffilla* are found in the same organs of the various molluscs.

The best season of the year in which to secure *G. gemellipara* at Woods Hole is during August, from the 10th to the 20th of the month. Specimens may be obtained prior to this, but they are usually immobile individuals which contain numerous young that have liberated themselves from their capsules and are swimming about in the mesenchyme. Such material is valuable for obtaining very young animals. On July 5, 1911, several of these exhausted mothers were placed separately in hanging drops of the fluid taken from the mantle cavity of *Modiolus*. The cover slip from which the drop was suspended was placed above the cavity of a hollow ground slide and sealed with vaseline. In this way the specimens could easily be studied under the microscope. On the following day it was noted that most of the young had ruptured the wall of the mother and were swimming about in the drop. In one case the escape of the young was actually observed. Young animals secured by this method can be kept alive without much trouble for about two days, and undoubtedly would live longer if proper care were taken. However, it was found unnecessary to obtain material for study in this way after forty-eight hours, for the washings of *Modiolus* yield many young specimens that correspond in size to these two-day old worms.

An interesting periodicity in the reproduction of *G. gemellipara* occurs at Woods Hole. From the 20th to the 25th of June (1911), shortly after the writer arrived there, specimens were secured in considerable numbers, but from this date until about the 10th of August it was extremely difficult to obtain material, although molluscs from many different regions were examined. From an entire bucketful of the *Modiolus* not more than a dozen would be secured, and these were either very young, sexually immature animals, or very large individuals which were about on the point of undergoing degeneration and freeing their young. About the middle of August, both in 1910 and 1911, *Graffillæ* were secured without difficulty, but from the 25th of the month until the 12th of September, when I left Woods Hole, they were extremely scarce. From this it would seem that there are two summer periods of rapid multiplication, one in June and the other in August; and possibly a third period occurs in October. Linton reports that Coe found *Graffilla* in abundance at New Haven during the month of October.

At no time does one find *G. gemellipara* in such numbers as reported by some of the writers on the other species of the genus. Jameson, '97, states that from four to several dozen individuals of *G. buccinicola*, which is parasitic in the kidneys of *Buccinum undatum* and *Fusus antiquus*, are found in every specimen of the two molluscs.

II. Structure of the Reproductive Organs.

The reproductive organs of this *Graffilla* are difficult to make out, both on account of the viviparous method of reproduction as well as on account of the variability in the development of the different parts. *G. gemellipara*, like certain other members of the genus, exhibits successive hermaphroditism, but the case is not so extreme as that described for *G. buccinicola* by Jameson, '97. The male organs develop first and upon reaching their maturity at a comparatively early period in the post-natal life, in part atrophy, and are then followed by the development of the female organs.

The male organs consist of the following parts: (1) a pair of testes which lie just posterior to the pharynx, one on each side

of the median line somewhat below the central axis of the animal (Fig. 1); (2) two very delicate, short sperm ducts which place the gonads in communication with the seminal vesicle; (3) a seminal vesicle, which is a rather large pear-shaped sac situated just below the genital pore; and finally, a plug-like penis arising from the pointed, ventrally directed end of the seminal vesicle. In one of the clearest specimens secured each sperm duct is seen to arise from the posterior median corner of the testis and to pass inward to the anterior face of the seminal vesicle, meeting the latter at about the dividing line between its upper, bulbous portion and the smaller lower part. The penis when contracted is extremely difficult to make out, and since in mounted preparations this condition is almost invariably met with, not many of the details of the organ were studied. The penis when extended of course protrudes into the common atrium, which in turn communicates with the exterior by means of the small genital pore. The pore lies in the median ventral line at a point situated about one third the distance from the anterior end of the body.

In large individuals the testes are seldom found, and when present are mere degenerating fragments. The seminal vesicle, however, persists at least until a late period of the post-natal life, but in many animals becomes reduced in size. The penis also degenerates sooner or later. During this period of degeneration of the male organs the female reproductive structures gradually make their appearance. One occasionally meets with specimens in which the transition from the "male" to the "female" state is seen, and from such individuals most of the important points concerning the female organs can be made out.

In the typical "female" condition the seminal vesicle is always present, though as stated above it may become greatly reduced in size, and the atrium with its genital pore still persists. Just back of the seminal vesicle and dorsally the atrium gives rise to a small diverticulum, which both from its position and character suggests its homology with the receptaculum seminis of the other members of this genus, although in the two clearest cases coming under the observations of the writer this vesicle contained no spermatozoa (Fig. 2). If this interpretation is correct then the receptaculum seminis is in this species clearly a degenerate structure.

Posteriorly the atrium is directly continuous with an enlarged, rather thick-walled uterus, which in turn gives rise to a duct-like structure that extends backwards and upwards (Fig. 5, *u*). At the point where these two parts join, the uterus receives the small ducts of the many unicellular shell-glands (Fig. 1, *s*).

Towards its distal end the uterus bifurcates, sending a branch to each of the bilaterally arranged ovaries (Fig. 3). The bifurcated part of the uterus serves as a receptacle for spermatozoa —a condition that is not entirely unique for this species—and also performs the function of insemination. On account of the backward and upward course taken by the uterus, the two distal parts come to lie just below the ventral surface of the intestine, at a place slightly posterior to the middle point of the body (Fig. 5).

The development of the uterus has not been studied and I can not therefore state with certainty the exact nature of this organ. Slightly posterior to the point where the proximal and distal parts join the duct is frequently very indefinite and difficult to trace. This, together with the fact that small yolk cells are frequently found within its cavity (Figs. 4, 5) has led the writer to believe that the distal part of the uterus is the product of fusion between the ducts coming from the reproductive glands and therefore should probably be called the oviduct.

The female reproductive glands consist of a paired "germarium" and a paired "vitellarium," the two glands on each side being so closely associated that the compound structure might properly be termed a "germ-vitellarium." The ovarian portion occupies the anterior part of the body, while the yolk glands occupy the posterior half mainly.

The clearest idea of the relation of these various parts to each other and to the reproductive ducts can best be gained in a study of horizontal sections which pass just below the ventral side of the intestine. In such sections the ovary on each side is seen to begin slightly anterior to the seminal vesicle, and to increase gradually in diameter in passing backwards until it reaches the region occupied by the distal end of the uterus. Here it spreads as a fan-like structure, with the inner margins of the ova converging to meet the tip of the uterus (Fig. 4). In composition

the ovary is made up of flattened cells, and one might compare it to a rouleau of coins of gradually increasing size, the smallest being located at the anterior end. The larger cells of the ovary are produced by the absorption of nutritive materials from the vitelline cells, in a manner that will be described in the next section.

The vitellarium is an extensive organ, and in the posterior half of the body almost completely envelops the intestine (Fig. 6). In the early stages of its formation the cells are very similar to those of the ovary, and even in the definitive condition their nuclei have the characteristics of ovarian nuclei. The ovarian and vitelline cells are in very close association at the middle region of the body, and for some little distance anterior to this the ovary is overlaid by the yolk cells.

III. Early Development.

1. *Nutrition of the Ova and the Formation of the Egg-capsule.*— In order to be able to understand clearly the manner in which the ova are nourished and the egg-capsule is formed it is necessary to call attention to the characteristic condition in *Graffilla* of the duplexity of embryos in each capsule. In all of the older stages the two embryos are surrounded by a very thin transparent membrane or shell inside of which the two ciliated individuals may move about each other with considerable ease. In late cleavage, or indeed in any stage of segmentation, this thin shell in the strict sense of the word does not exist, though the outermost portion of the yolk is of a consistency such that it serves the purpose of a shell, and out of this surface layer the true shell doubtless differentiates. During the cleavage stages it is seen that a considerable mass of yolk surrounds the two embryos (Fig. 19). The two embryos may be either close together, with only a very thin intervening layer of yolk, or widely separated and situated at the extreme opposite ends of the capsule (Figs. 2, 3). In either event the most pertinent question that one can raise is how the two embryos have come to exist within the same yolk mass.

As we have pointed out in the preceding section, the ovaries are at their posterior ends somewhat closely approximated on

the ventral side of the intestine, and are intimately associated with the yolk glands, being surrounded on the dorsal and posterior aspects by them. In a longitudinal section of almost any individual in the egg-producing stage one can observe that the ova are at their upper margins absorbing yolk from these glands, and while the nutritive process may involve the ova of one half of the ovary, yet it is much more conspicuous in the posterior third of that organ (Fig. 9). At the extreme end of the ovary the absorption goes on with great rapidity, the ova soon becoming gorged with nutritive material. In consequence of this rapid growth certain retrogressive changes involving the cell membranes separating contiguous ova frequently make their appearance. As a result two or even more nuclei may come to lie within a common yolk mass, which occupies the extreme tip of the ovary (Figs. 9, 10). In other words, a syncytium is formed here. In the vast majority of cases only two ova are involved so that the usual picture displayed in this region represents a binucleated yolk mass (Fig. 15).

It should be noted here that in this peculiar method of nutrition we have a mechanism alone adequate to account fully for the reason why two embryos are habitually borne within a single capsule. Just why two should appear is difficult to answer. As a matter of fact, however, two are not always present, for as Linton has pointed out capsules are sometimes seen with three embryos, and a few cases were noted by him in which only one embryo is surrounded by the envelope. Furthermore, in the figure of Nicoll referred to above, two capsules containing three embryos each are clearly shown. In my own material several cases of "triplets," including one with undivided eggs, have been observed, as well as several with one embryo each. While in the light of these facts the twin condition in *Graffilla* loses much of its apparent significance, yet its appearance in the great majority of cases made it necessary to undertake a careful study of the histogenesis of the ovary in order to see if any mechanism, other than that of the breaking down of intervening membranes, could be discovered that would explain a potency to gemelliparous reproduction on the part of that organ. At first it seemed probable that a binucleated ovum was produced somewhere in the oögonial history. A diligent search in the ovary fails to

reveal any binucleated ova, except of course at the extreme tip, nor has the slightest evidence been secured of nuclear divisions either mitotic or amitotic throughout the entire length of a fully matured ovary. We are therefore forced to the conclusion that what we have described in connection with the absorption of yolk furnishes the key to the twin condition in *Graffilla*. It can not be argued that the breaking down of the membranes is only apparent and therefore an artifact produced by reagents, for it has been observed in preparations made from material preserved in a dozen different fixing fluids, and followed by as many different stains. However, not in all cases do the two contiguous ova lose their intervening membranes, but some become completely surrounded by vitelline cells, which through a process of disintegration form the yolk mass of the definitive capsule (Figs. 7, 16). In such cases the two ova do not lose their "individuality," and a subsequent reorganization of new membranes about the two nuclei will not take place. Considerable evidence has been secured which indicates that these two methods of capsule formation are but the extremes of one and the same process.

Throughout the entire history of yolk absorption many interesting changes, involving both the nucleus and cytoplasm, are seen, but we can not deal with all of them here. Our attention must therefore be directed to those that seems to us to be most important.

In Fig. 14 is represented a pair of nuclei lying within a single membrane. The lower of these is immediately surrounded by a layer of finely granular protoplasm, about which one can trace another very delicate, but nevertheless distinct, membrane. This condition has been observed in a number of ova, and may begin before the binucleated stage is reached, that is, in ova situated from two to six cells from the tip of the ovary. I have not been able to demonstrate the universality of this membrane, and I am therefore inclined to regard it as the intra-cellular or intra-vitelline membrane that is sometimes laid down about the ovarian nucleus. It may be that in *Graffilla* it marks the beginning of the segregation of the protoplasm from the yolk, and is therefore the first step in the reorganization of a cell about each of the nuclei in the capsule.

In Fig. 9 is seen the last trace of the intracellular membrane in a binucleated mass that is about ready to be freed from the ovary. It is possible of course that the faint line about the large nucleus is not an intra-cellular membrane, but only the original cell-wall which has become much attenuated through the absorption of yolk by the ovum. This figure is of further interest in that it demonstrates with remarkable clearness the manner in which the yolk is absorbed by the ova. At the extreme end of the ovary the process is at its height, and one can actually observe the configuration of the streams of food material extending from the vitelline cells to the larger nucleus. This is particularly true in the pseudopodial-like structure in the upper median portion of the figure. On the extreme right, near the section of the tip of the second nucleus, the yolk cells are directly open to the ova. It is not quite clear as to what extent the yolk cells participate in the formation of the mass of yolk surrounding the eggs, aside from merely giving up their nutritive materials; but that they do assist in this formation is abundantly proved in those capsules the yolk contents of which show many degenerating nuclei of vitelline cells. In some cases these fading nuclei form a complete row just below the surface of the capsule.

Some half dozen cases have been found in which the ovum apparently does not become surrounded by any considerable amount of yolk, but after absorbing a small amount of food material is set free from the ovary. These single naked eggs float about in the parenchyma and probably never succeed in producing embryos (Fig. 13).

Some time prior to the liberation of the ova from the ovary and the yolk-gland, the ovarian nuclei undergo marked changes. During all of the preceding oögonial history the nucleus possesses that characteristic coarse network of chromatin extending throughout the nucleoplasm, and a very large, deeply staining nucleolus (Fig. 9); but during the last stages of yolk absorption the chromatin network becomes more or less indistinct (Fig. 7), finally disappearing altogether, and in its stead a finely granular condition of the chromatin appears. At the same time the nucleolus stains less intensely and soon becomes very irregular in outline (Fig. 10).

It is necessary to mention only briefly the manner in which the "ovulation" takes place. By the time the absorption of yolk has reached the point seen in the case of the ova on the right of Fig. 9 the formative capsule may be said to be practically independent of any ovarian connections, and it only remains for the capsule to be freed from the vitellarium. However, its attachment with the yolk glands persists for some time after this, even indeed until the two eggs reorganized, if reorganization is necessary. In Fig. 10 is a capsule just about ready to be set free into the parenchyma; most of the yolk cells have yielded up their food contents to the capsule, and the region immediately surrounding its upper margin shows only delicate strands connecting it with a few of the remaining nurse cells. Shortly following this period the strands are severed and the capsule rounds up, and as the whole structure is pushed about in the parenchyma by the movements of the mother worm the eggs undergo development.

Up to the present we have been using the term "capsule" to mean the whole yolk mass surrounding the two eggs; and we must now consider briefly the formation of the thin capsule or shell, by which we mean the membrane containing the two ciliated embryos of the later stages. Since the eggs with their follicular layer of yolk do not enter the uterus, it is not probable that any of the secretions from the unicellular shell-glands reach the eggs and thus take part in the formation of the shell, as occurs in the case of oviparous forms. I have not followed all of the steps in the formation of the shell, but it has been observed that as development proceeds the outermost layer of the yolk, which at first is very plastic and yields readily to any obstruction in the parenchyma, gradually becomes more resistant, finally taking on the thin elastic character met with in all of the advanced stages. It is probable that the shell is in part the product of the parenchyma.

It remains to say a word about the "reorganization" of cells in those cases in which the membrane in part or completely disappears from the two ova. Even in the extreme cases it is doubtful whether the cytoplasmic part of the cell becomes indiscriminately associated with the yolk portion of the capsule.

This part of the study has furnished many difficulties, because of the fact that the capsule at this particular stage is very plastic and hard to fix properly. Only a few cases of good fixation have been secured; and in one of the clearest of these the nuclei are seen to be surrounded by a finely granular protoplasm, about which a membrane must later be secreted.

2. *The Aborting Spindle.*—The study of maturation and fertilization was made difficult by the presence of a spindle which appeared in the egg some time before the egg capsule was set free into the parenchyma. On account of its large size the spindle was at first taken to be that of the first cleavage, but inasmuch as the first division of the fertilized egg results in cutting off a small micromere, it soon became evident that this interpretation was incorrect. Furthermore, in the eggs in which the large spindle appeared the most diligent search failed to reveal any polar bodies. When this fact once became fully established it was evident that we had in *Graffilla* a display of that remarkable phenomenon of a "disappearing" or "aborting" spindle, first discovered by Selenka, '81, and to our knowledge of which Wheeler, Gardiner, and others have contributed.

Selenka's discovery was made in connection with his work on the polyclad *Thysanozoön Diesingii.* He describes the aborting spindle as appearing in the uterine eggs. After the egg has reached its full growth, the germinal vesicle begins to make preparations to divide in the typical manner; the chromatin forms a spireme, the achromatic spindle with its two centrosomes appears, and the chromosomes pass into the equatorial-plate position. At this point the process stops, and the nucleus returns to a resting condition. Subsequently the egg throws off two polar bodies, is fertilized, and develops in the normal manner. Inasmuch as the yolk granules are evenly distributed throughout the egg at the beginning of this peculiar phenomenon and are collected about the astral centers at its close, Selenka supposes that the function of the aborting spindle is to mass the granules at the center of the egg. But this interpretation fails to explain the appearance of the spindle in those eggs in which a collecting of the granules about the astral centers does not take place, as both Lang and Wheeler have observed.

Lang, '84, next noted the aborting spindle in several polyclad eggs, and figures it in the uterine egg of *Thysanozoön Brocchii.*

Wheeler, '94, describes briefly the appearance of the uterine spindle in the eggs of *Planocera inquilina*, a polyclad inhabiting the branchial chamber of *Sycotypus canaliculatus*, but does not attempt to work out the details of the process. He also noted the spindle in the eggs of the acœlan *Polychœrus caudatus.*

Gardiner, '95 and '98, working on the latter species came to the conclusion that the aborting spindle is abnormal, representing the first cleavage spindle of eggs retained too long in the uterus of an animal kept under abnormal conditions. His point does not seem to be well taken, as Surface, '07, has shown in his work on *Planocera.*

The last reference to the aborting spindle that we may note is that of L. von Graff, '82, in his monograph on the Rhabdocœlida. Von Graff, although making no reference to the spindle in the text, clearly figures one in the uterine eggs of *Aphanostoma diversicolor* and *Cyptomorpha saliens.*

In our species, *G. gemellipara*, the aborting spindle appears in the eggs some time before the freeing of the egg-capsule from the vitellarium. The spindle is really anticipated long before all of the yolk is laid down about the two eggs, as can be seen in Fig. 19. In many respects the spindle is truly remarkable, not only on account of its great size, but also for the reason that frequently the chromosomes do not appear upon it. One of the clearest cases that has come under my observation is shown in Fig. 17. This is an especially well preserved egg, yet one can not detect the slightest trace of chromosomes in the cell. However, it is probable that the chromatin is represented by some of the central spindle fibers, which are quite thick but do not take the stain well. This is most certainly the case in some eggs in which very delicate chromatin threads among the spindle fibers can with difficulty be made out.

Sometimes the chromatin is in the form of chromosomes, which however are not located on the spindle. In Fig. 18 is shown such a case. Here the large conspicuous spindle is itself free from chromatin, but among the astral rays of one end are four chromosomes, which are of interest not only because of their peculiar

position, but also because they are apparently bivalent. They are not tetrads in shape, as in the characteristic condition of the first maturation, yet that they are the egg chromosomes and not those of the sperm is evidenced by the fact that the sperm is located in another part of the ovum.

The peculiar behavior of this karyokinetic figure is not confined to the chromatin; the centrosomes frequently present unique conditions. It is not uncommon to find the centrosome at one or both ends of the spindle undergoing division, but this would not be striking—since in many germ cells, both male and female, a precocious division occurs—were it not for the fact that at one end the axis of the two centrosomes is at right angles to that of the spindle, while at the other end it is simply a continuation of the spindle axis. The precocious division of the centrosome frequently results in the formation of a double aster.

I have not been able to follow with certainty all of the subsequent steps in the history of this spindle, but the end result in all cases would seem to be a return to a sort of resting stage on the part of the nucleus. It differs from the corresponding stage of *Thysanozoön Diesingii*, in that the nucleus instead of being a large vesicle, appears in the form of four vesicles, one for each chromosome (Fig. 19). These may be more or less grouped together or widely separated, but they later come together and fuse, producing a lobulated nucleus which retains this condition until the onset of maturation (Fig. 21). It will be seen from this rather brief account that the only function which one might assign to the aborting spindle in *G. gemellipara* is that of scattering the chromosomes in the form of vesicles; but since these are later collected together into a single vesicle before maturation, it is difficult to attach any real significance to this whole peculiar phenomenon. Inasmuch as several odd conditions have been observed, both in the centrosomes and the chromosomes, it is not at all improbable that the aborting spindle is an abnormal display. But it can not be the result of placing the animals under unfavorable conditions because the spindles are found in worms killed immediately upon their removal from the mollusc.

It should be pointed out here that *Graffilla* is not a favorable

form in which to work out the history and significance of the aborting spindle, for owing to the viviparous mode of reproduction prevailing in this species it is quite impossible to secure a complete series of stages showing the different steps. One can not be at all certain that it occurs in every egg, though the frequency at which it is met would indicate that it did. Nevertheless, it would seem that some rather important function should be assigned to the aborting spindle; for its appearance in some dozen different species of flat worms must exclude it from the category of abnormal behavior. It is therefore hoped that an opportunity may be offered to work out its history in detail in a favorable form, such as one of the oviparous species from which a series of stages can be secured from the uterus.

3. *Insemination.*—By insemination is usually meant the act of introducing the spermatozoa into the egg. In *Graffilla* the process occurs during the last stages of yolk absorption while the formative capsule is still attached to the ovary, and consists in the introduction of spermatozoa into this capsule. The inseminating organ is the modified, or bifurcated part of the uterus. In Fig. 6 is shown a beautiful case. The section passes through the distal end of the uterus, and the left-hand lobe of that organ, filled with spermatozoa, is in direct contact with the binucleated capsule. Any number of similar figures can be demonstrated in the preparations, so that no doubt can exist regarding the interpretation which we have placed upon such pictures. It would seem that the uterus took an active part in the process of insemination. Linton reports an observation which points to the same conclusion.

This method of insemination must necessarily permit a number of spermatozoa to get into the capsule, but owing to their small size they are soon lost among the yolk granules, so that an enumeration of them is impossible. So far as one can tell the sperms do not at first invade the immediate neighborhood of the two nuclei, but remain in the peripheral portion of the capsule, and later penetrate the eggs a short time before the beginning of maturation.

4. *Maturation.*—As in the case of all ova accompanied by the process of fertilization, those of *Graffilla* throw off two polar

bodies. The first maturation follows immediately upon the fusing of the chromosome vesicles produced by the aborting spindle, and at the time it occurs the sperm is already present in the egg (Fig. 21). The demonstration of maturation as taking place simultaneously in the two eggs within the same capsule is the most cogent proof we can offer against the idea that this animal exhibits polyembryony; because if this is a fact, each egg must subsequently be fertilized before it could develop, and that would at once remove the case from the category of polyembryony; and even though no other proof could be offered, such as we have given in connection with the section on the formation of the capsule, this would be sufficient to establish our main contention. As a matter of fact we have found two very clear cases in which each of the two eggs is undergoing maturescence.

The egg in one of these shows the first maturation spindle in the anaphase (Fig. 20). The spindle is extremely large and has at each end a large aster with very conspicuous centrospheres, in the lower of which is a single centrosome and in the upper of which are two centrosomes. The sperm head, already showing signs of its transformation into a pronucleus, lies near the lower aster. Between the upper pole of the spindle and the egg-membrane is a clear space due to a depression in the egg at this point. In a slightly later stage the egg elongates in the direction of the long axis of the spindle, taking on an appearance much like that of a pear, with the smaller end representing the animal pole. A very large polar body is then cut off, and the mate to this egg fortunately shows this process going on (Fig. 24). Since the first cleavage division results in producing a micromere of about the same size, opportunity is afforded for confusing this cell with the first polar body, but the difference can easily be told if the chromosomes are in a condition that allows their enumeration to be made.

In the second case one of the eggs (Fig. 22, on the left) shows the maturation spindle in prophase with four distinct tetrads, and the other cell a polar view, in which only three chromosomes appear. I have been unable to find a fourth tetrad, and I therefore assume that it must have been destroyed by the knife.

Several eggs showing the first polar body just extruded have

been found. In a few of these the egg nucleus is in a resting condition, thus indicating that the second division may not follow immediately upon the first. However, I have not yet succeeded in finding the spindle of the second polar body division, but that a second polar body is thrown off is clearly shown in at least one case (Fig. 25). Here the constriction of the second polar body has just been completed, while the first polar body having undergone division is in the process of disintegration. The rapid disappearance of the polar bodies immediately after they are given off has added to the difficulty of studying their formation, as well as to the study of the formation of the first micromere.

Perhaps the most striking feature of maturation on *Graffilla* is the large size of the first polar body. This is not surprising; for it is not uncommon for a large polar body to be given off in the eggs of certain flat worms. It was in the egg of a turbellarian, *Prostheceræus*, that Francotte, '97, discovered the interesting fact that the first polar body may be nearly as large as the egg itself, and may occasionally be fertilized and develop into a small gastrula, after having first formed a small polar body like the second one of the egg.

5. *Fertilization and the First Cleavage.*—Fertilization follows almost immediately upon the throwing off of the second polar body. I have found no exceptions to the rule that only one spermatozoön enters the egg. The sperm penetrates the egg in the vegetative hemisphere (Figs. 20, 21, 24), and passes toward the center where it remains while the polar bodies are being given off. The sperm nucleus then moves to a point near the animal pole where the copulation of the two pronuclei occurs (Fig. 26).

The first cleavage is unequal and results in cutting off a micromere at the animal pole. Any number of first cleavage spindles have been observed, and they are all characterized by having eight chromosomes, and by having centrosomes which are much more conspicuous than those of the maturation spindles. In this as in all of the subsequent early cleavages, the nuclei enter into a "rest" stage immediately after the completion of the division; and instead of forming a single vesicle, the chromosomes

more or less retain their individuality, thus producing a number of small vesicles, some of which may, however, fuse together (Fig. 8).

IV. Some General Conclusions.

We find no evidence in *Graffilla* that the two embryos commonly found within a capsule are the product of a single fertilized egg. On the contrary, it is clear that they spring from two ova, which have become enclosed within a common envelope. In this respect our species does not present anything unusual; for while it is the rule among the rhabdocœles to have one embryo in a capsule, yet there are a number of well-known exceptions to this. In his excellent monograph on the turbellaria Von Graff, '08, has recently given a list (p. 2338) of these exceptions, which are as follows: *Gyratrix hermaphroditus*, *Provortex*, *Collastoma*, *Umagilla*, *Polycystis*, *Fecampia*, and *Monocelis lineata*, each has two embryos in a capsule; *Anoplodium*, 1–2; *Prorhynchus stagnalis*, 1–3; *P. balticus*, 6; *Graffilla*, 2–3; *Promesostoma marmoratum*, 4–7; *Dalyellia truncata*, *millportiana* and *viridis*, 4–12; *Plagiostomum vittatum* and *girardi*, 10–12; and finally, *Syndesmis*, 2–13. All of this goes to show that the facts which we have brought forward concerning the method of reproduction in *G. gemellipara* are entirely in harmony with what is known to occur in the other turbellaria. Even the manner in which the two ova become surrounded by nurse cells within the reproductive glands presents nothing new (unless it be in those cases in which the ova for a while lose their individuality). Furthermore, the habit of directly freeing the ova, with their nurse cells, into the mesenchyme is also seen in such forms as *Dalyellia viridis* and *Olisthanella obtusa*. In most forms in which two or more eggs are enclosed within a capsule the ova become surrounded by a common follicle of nurse cells before they pass to the uterus, where the shell or true capsule is usually secreted.

Some of the rather rare conditions seen in *G. gemellipara* are the indefiniteness of the reproductive ducts, the rudimentary state of the receptaculum seminis, the failure of the eggs to enter the uterus, and consequently the probable secretion of the shell by the mesenchyme. But all of these conditions are incident to the viviparous mode of reproduction. Linton suggests

that this viviparity may be seasonal and parallel with the production of summer eggs, as is known to be the case in some of the Mesostomata. Certain facts in *Graffilla* might seem to indicate that what we have described are the conditions peculiar to a period of summer egg production. Thus the thin shell is a distinctive characteristic of a typical summer egg (Subitaneier), and the well developed unicellular shell-glands suggest at least that these organs could function later, if the species entered upon a period of winter egg production (Dauereier). However, in the absence of any proof that winter eggs are produced, and in the light of the fact that several of the female reproductive organs show a rudimentary or degenerate condition, we are inclined to believe that what we have described is the exclusive method of reproduction in this species. The presence of shell-glands, of a rudimentary receptaculum seminis, and of an indefinite uterus and ducts, instead of indicating that the species could later produce winter eggs, may and probably do, signify the close relationship of this species to the other members of the genus in which these structures are functional.

Of the half dozen species of *Graffilla* described in the literature, *G. gemellipara* appears to come closest, in its general arrangement of organs, to *G. Muricicola*. It also shows some similarity to *G. buccinicola*, but differs primarily from the latter in having the genital pore situated further back on the body.

In conclusion, we should like to point out, as a result of our studies on this animal, the necessity of exercising great precaution in concluding that a given species exhibits polyembryony. Undoubtedly the phenomenon of polyembryony will, in the future, be found to be much more extensive than we have suspected; but before coming to any definite conclusions, the investigator should trace the development back to the fertilized egg.

LITERATURE.

Böhmig, L.

'86 Untersuchungen über rhabdocœle Turbellarien, I. Das genus *Graffilla*. Zeit. fur Wiss. Zool., Bd. 43.

Francotte

'97 Recherches sur la maturation chez les Polyclades. Mem. Cour. Acad. Sci. Belg.

Gardiner, E. G.

'95 Early Development of *Polychœrus caudatus* Mark. Journal of Morphology, Vol. 11.

Gardiner, E. G.

'98 The Growth of the Ovum, Formation of the Polar Bodies, and Fertilization in *Polychœrus caudatus*. Journal of Morphology, Vol. 15.

v. Graff, L.

'82 Monographie der Turbellarien, I. Rhabdocoele, Leipzig.

'08 Turbellaria. Bronn's Tier-reichs, Leipzig.

Hallez, P.

'87 Embryogénie des Dendrocoeles d'eau douce. Paris.

Jameson, H. L.

'97 Additional Notes on the Turbellaria of the L. M. B. C. District. Proc. and Trans. of the Liverpool Biol. Society, Vol. 9, pp. 160–178.

v. Ihering, H.

'80 *Graffilla muricola*, eine parasitische Rhabdocoële. Zeit. fur Wiss. Zool., Bd. 34.

Lang, A.

'84 Die Polycladen, Monographie. Fauna und Flora des Golfes von Naepel., Bd. 11.

Linton, Edwin

'10 On a New Rhabdocoele Commensal with *Modiolus plicatulus*. Journal of Experimental Zoölogy, Vol. 9.

Nicoll, W.

'06 Notes on Trematode Parasites of the Cockle and Mussel. Annals and Magazine of Natural History, Ser. 7, Vol. 17.

Schmidt, F.

'86 *Graffilla braunii* n. sp. Archiv fur Naturgesch., Bd. 1.

Selenka, E.

'81 Ueber eine eigentümliche Art der Kernmetamorphose. Biol. Centralblatt, Bd. 1.

Surface, F. M.

'07 The Early Development of a Polyclad, *Planocera inquilina* Wm. Proc. Acad. Nat. Sci. of Phil., Dec., 1907.

Wheeler, W. M.

'94 *Planocera inquilina*, A Polyclad Inhabiting the Branchial Chamber of *Sycotypus canaliculatus* Gill. Journal of Morphology, Vol. 9.

PLATE I.

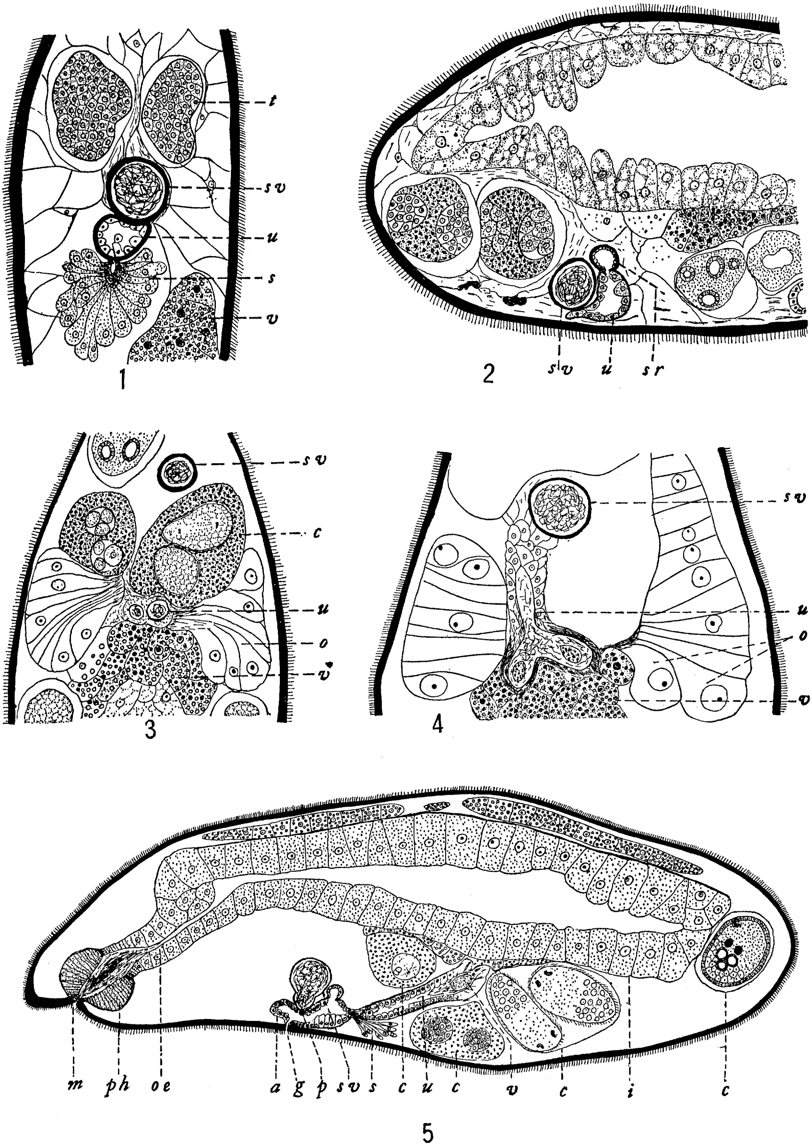
FIG. 1. Horizontal section of a young specimen, showing the testes (*t*), seminal vesicle (*sv*) which contains sperms, uterus (*u*), unicellular shell-glands (*s*), and the germ-vitellarium (*v*). × 222.

FIG. 2. Anterior half of a slightly oblique section from an adult individual. The uterus shows a distinct, but small diverticulum (*sr*) which in all probability corresponds to the receptaculum seminis of the other members of the genus. Note that the testes have disappeared. × 222.

FIG. 3. Horizontal section passing just below the intestine of a sexually matured individual. The section passes through the distal or bifurcated region of the uterus (*u*), which contains spermatozoa. *o*, ovary; *c*, capsule containing two eggs, one of which is giving off the first polar body; *v*, vitellarium. × 222.

FIG. 4. Horizontal section of another sexually matured animal, but which passes at a slightly lower level than the preceding. It shows clearly the bifurcated region of the uterus; and also the relationship existing between the uterus, ovary and vitellarium. × 222.

FIG. 5. A longitudinal median section (slightly schematized) of a rather old individual. It shows an advanced stage of the "female" condition. *m*, mouth; *ph*, pharynx; *oe*, œsophagus; *a*, atrium; *g*, genital pore; *p*, penis; *sv*, seminal vesicle; *s*, unicellular shell-glands; *u*, uterus; *v*, vitelline cell in uterus; *c*, capsules containing embryos; *i*, intestine. × 117.



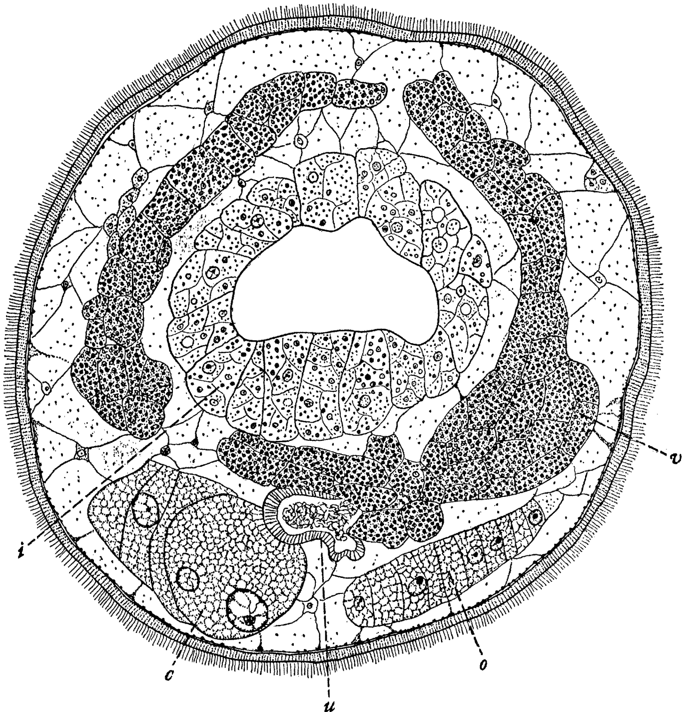
J. T. PATTERSON.

PLATE II.

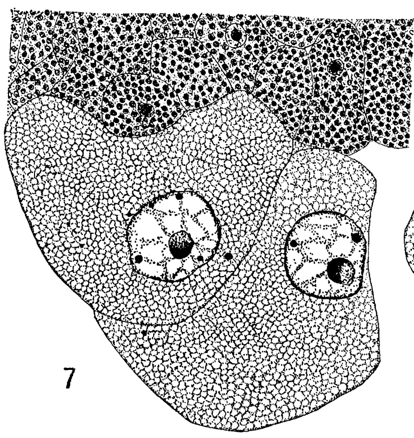
FIG. 6. Transverse section taken through the region of the tip of the uterus. × 381.

FIG. 7. Two ova that are beginning to be surrounded by vitelline cells preparatory to the formation of a capsule. × 784.

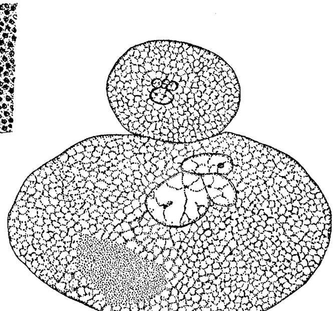
FIG. 8. The two-celled stage, showing a micromere and a macromere. × 740.



6



7



8

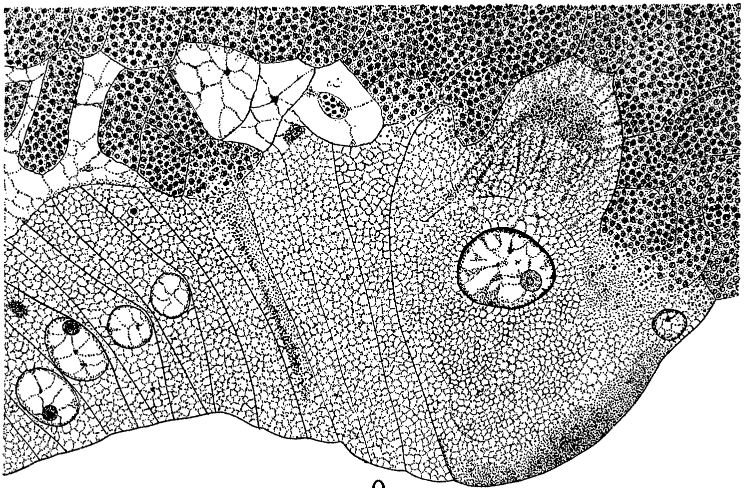
J T. PATTERSON.

PLATE III.

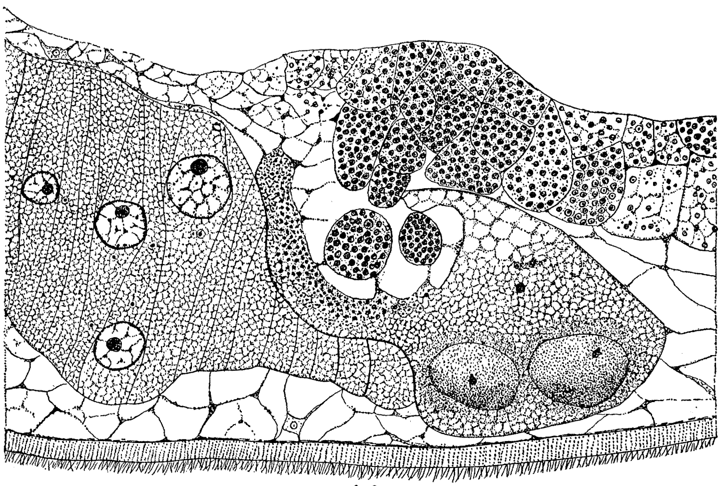
FIG. 9. The posterior half of an ovary which shows the process of yolk absorption. On the right a capsule is being formed about two nuclei. × 650.

FIG. 10. A later stage in the same part of another ovary. Note that the two nuclei are immediately surrounded by a finely granular protoplasm. × 650.

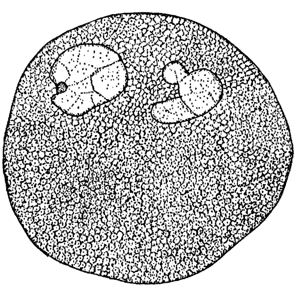
FIGS. 11 and 12. Two eggs from the same capsule. This represents the condition shortly after the disappearance of the aborting spindle. The nucleus is in the form of faintly staining vesicles which in part are fused together. × 812.



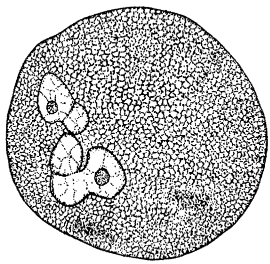
9



10



11



12

PLATE IV.

FIG. 13. Two naked ova that have not become surrounded by a capsule. Such eggs apparently float about in the parenchyma, but probably never produce embryos. × 543.

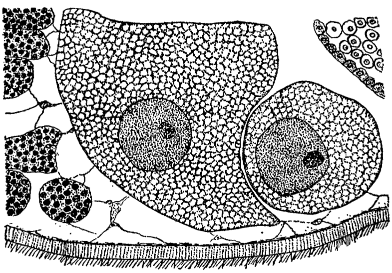
FIG. 14. A binucleated capsule in which the lower nucleus is surrouned by an intravitelline membrane. × 798.

FIG. 15. A binucleated capsule. × 543.

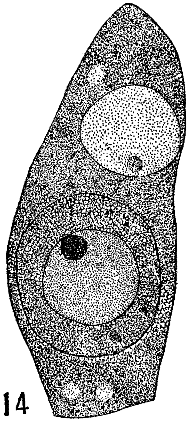
FIG. 16. Two ova completely surrounded by a follicular layer of vitelline cells. Only a part of one of the eggs is seen in the section. × 543.

FIG. 17. A typical case of an aborting spindle. Note that chromosomes are absent from the spindle. × 798.

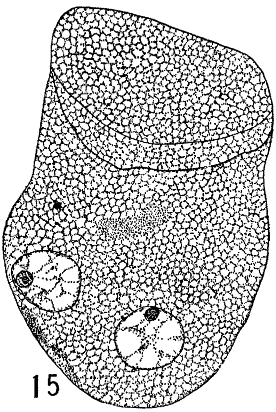
FIG. 18. Another example of aborting spindle, in which the chromosomes are located among the rays at one end. × 798.



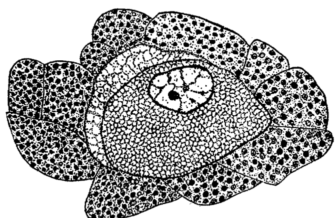
13



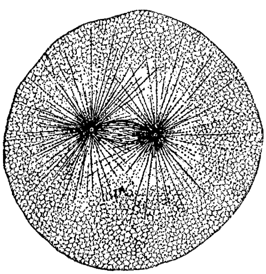
14



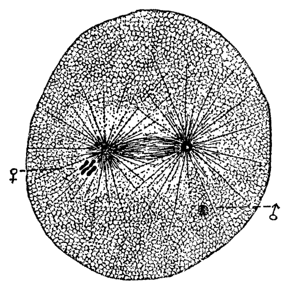
15



16



17



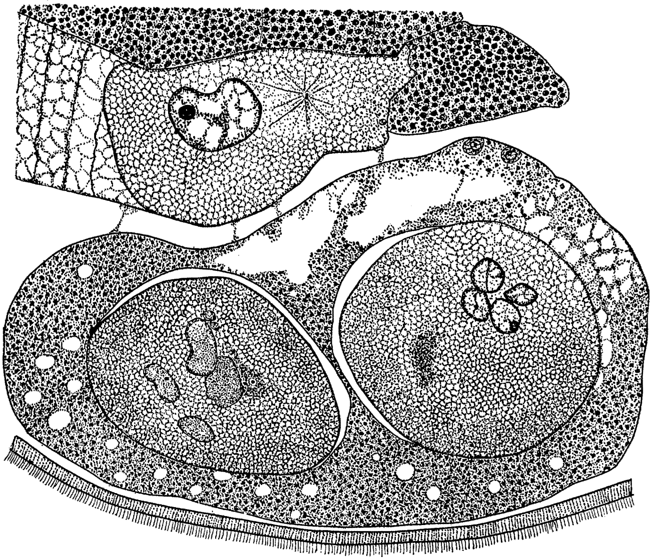
18

J. T. PATTERSON.

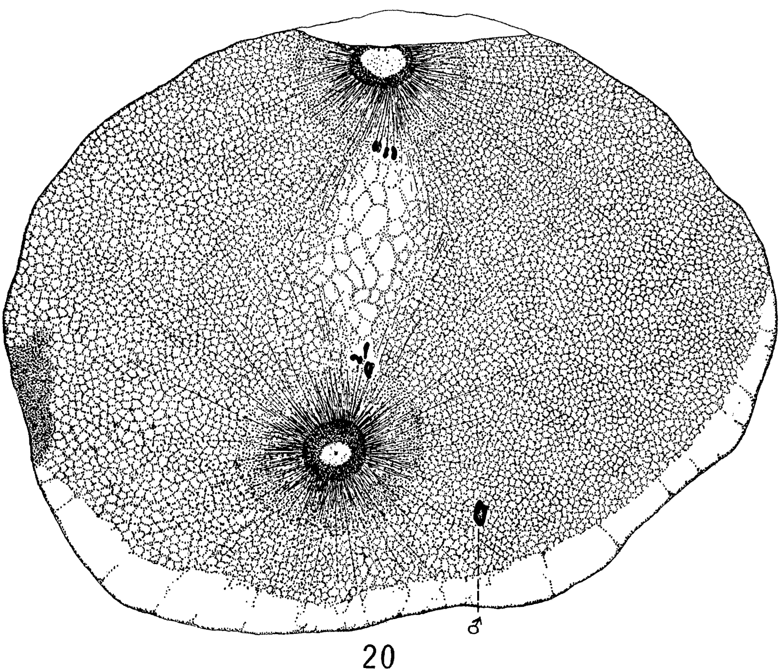
PLATE V.

FIG. 19. This shows a capsule about to be set free into the parenchyma. The eggs exhibit the condition which immediately follows the disappearance of the aborting spindle. Each egg has four chromosome-vesicles, and in the one on the left the centrosome is present. Lying just above this newly formed capsule is another in the process of formation. Only one of the ova shows in the section, and in it the centrosome has divided and the aster is present, thus anticipating the forthcoming aborting spindle. $\times$ 993.

FIG. 20. The anaphase stage of the first polar spindle. $\times$ 2,394.



19



20

J. T. PATTERSON.

Plate VI.

Fig. 21. An ovum shortly before the formation of the first polar body. The nucleus is the product of the fusion of the chromosome-vesicles of a stage like that in Fig. 19. The section passes through but one of the two ova in the capsule. In most of the capsules of this period the protoplasm of the eggs contracts in the reagents more than does the surrounding vitelline material, thus producing a clear space between the two materials. × 543.

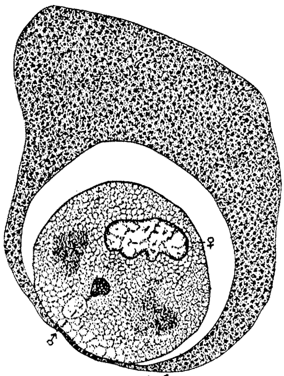
Fig. 22. A capsule in which both eggs are undergoing maturation at the same time. × 543.

Fig. 23. Two of the tetrads from the preceding figure. × 2,394.

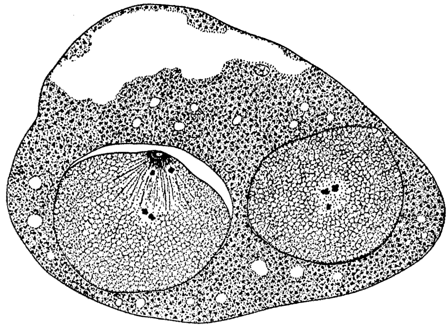
Fig. 24. The cutting off of the first polar body. This egg is a mate to the one shown in Fig. 20. × 798.

Fig. 25. This stage shows the close of maturation. The first polar body has undergone division and is disintegrating. × 543.

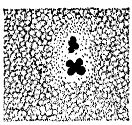
Fig. 26. Fertilization stage. × 543.



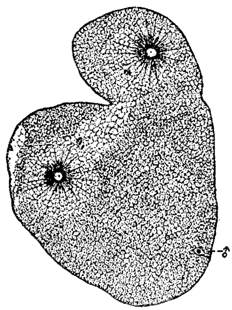
21



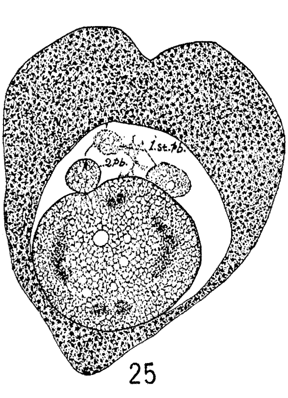
22



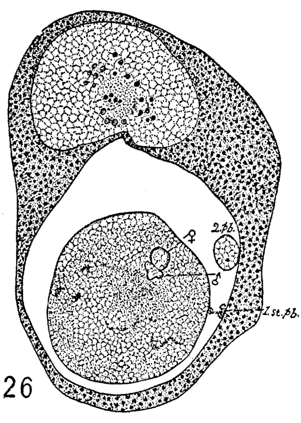
23



24



25



26

J. T. PATTERSON.